INVITRO STUDY ON ANTI-CANCER ACTIVITY OF BLUE BERRY EXTRACT OF COLON CANCER CELL'S

Dissertation submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY

Chennai-600032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
PHARMACOLOGY

Submitted by

REG. NO: 26103392

Under the Guidance of

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APRIL -2014

DECLARATION

I hereby declare that that this thesis work entitled as **"*INVITRO* STUDY ON ANTI-CANCER ACTIVITY OF BLUE BERRY EXTRACT OF COLON CANCER CELL'S** submitted to The Tamilnadu Dr. M.G.R Medical University ,Chennai was carried out by me in the Department of Pharmacology , Ultra College of Pharmacy ,Madurai under the valuable and efficient guidance of **Mr.SRIDHARM.Pharm,** Department of Pharmacology, Ultra College of Pharmacy , Madurai during the academic year 2012 - 2013. I also declare that the matter embodied it is a genuine work and the same has not performed the basis for the award of any degree, diploma, associateship,fellowship of any other university or institution.

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CERTIFICATE

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during the academic year 2012 – 2013.

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EXAMINERS:

1.

2.

PLACE: MADURAI

DATE

The work done is dedicated to all those who have contributed to the development of pharmacology

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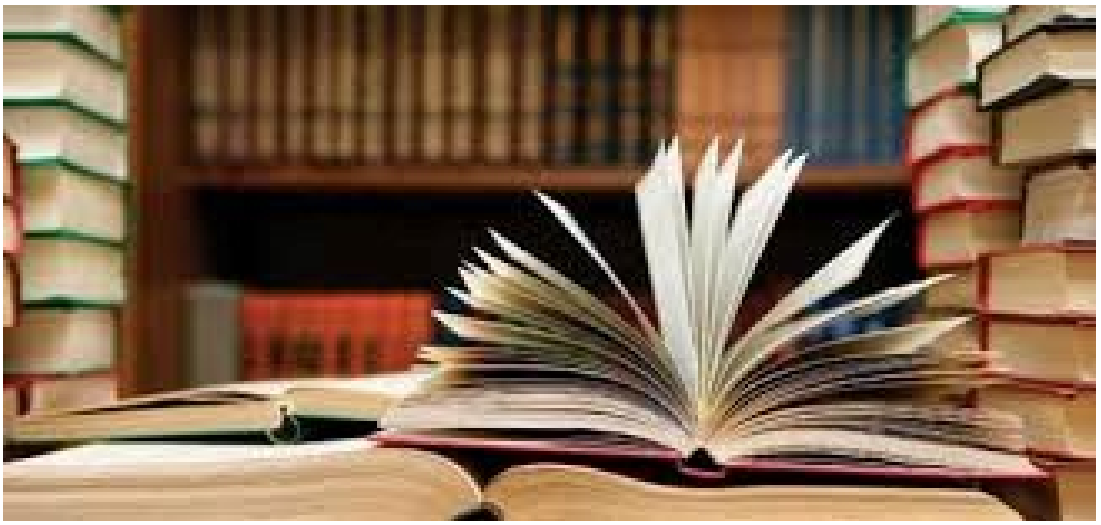

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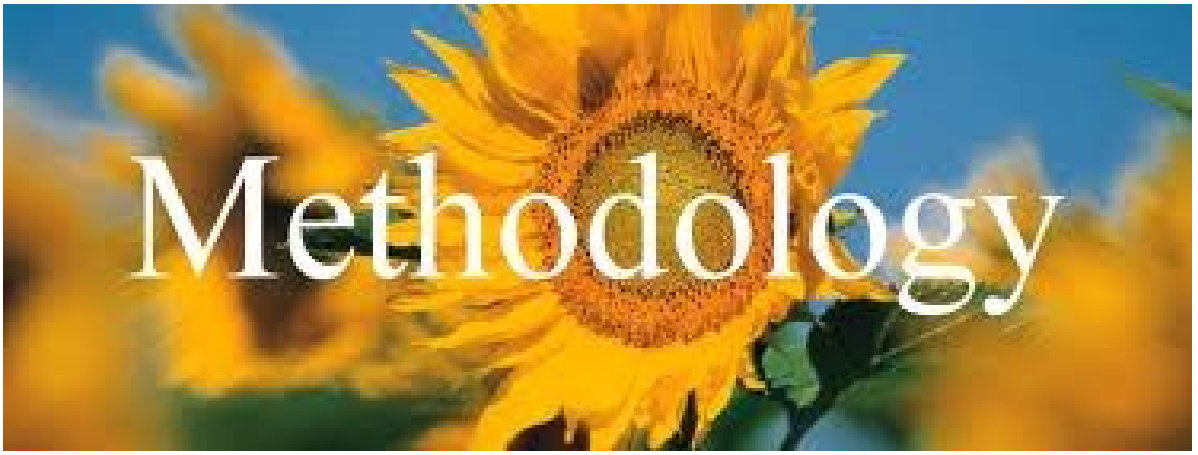

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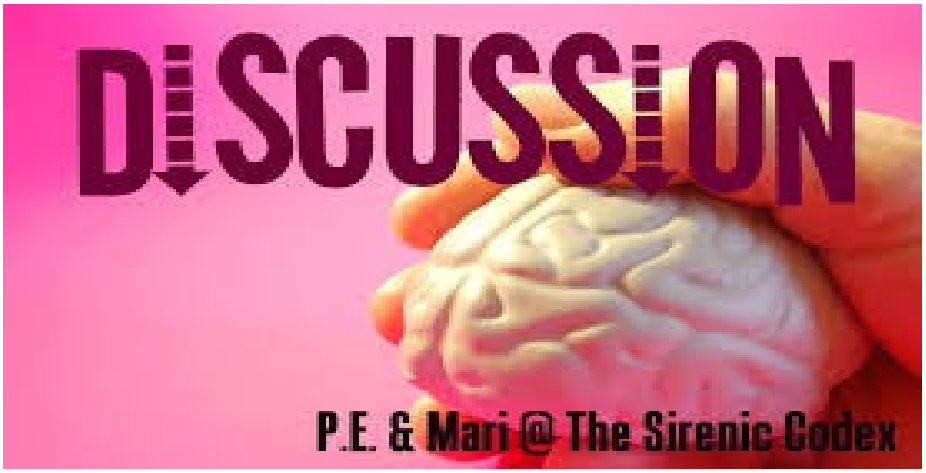

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"The expressivity of words looses significance when we search for an appealing sentence of gratitude and obligation, since acknowledgement is the only part of dissertation which lacks guidance".

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CONTENTS

INTRODUCTION

1.a. COLON CANCER:

The cancer which occurs in the large intestine or the rectum is called colon cancer. The end of the colon is called the rectum.

There are various other types of cancer which effects the colon such as lymphoma, carcinoid tumours, melanoma and sarcomas. But such type of cancers occur very rarely. Every colon cancer starts in the lining of the colon. There is no particular cause of the colon cancer. But this cancer starts as benign polyps which then gradually leads to cancer. Colon cancer is considered to be a malignant tumour and the colon cancer ranks second. The colon cancer is nowadays increasing in china. The etiology and pathogenesis is not clear. (A Rietveld; 2003)

Most colon cancer mainly due to the lifestyle and with the increase of the age and is related to some genetic disorders. It mainly starts in the lining of the colon in the stomach and when left untreated it spreads to the wall. Restricted colon cancer are usually detected through colonscopy. Cancers which are mainly restrained to the wall of the colon are usually curable with the help of surgery, while those cancers which has spread throughout the body cannot be cured and the particular persons life can be extended just through chemotherapy. Colon cancer is said to be the normally detected cancer in the world and is said more common in developed countries. Studies detected that in 2008 1.24 case of colon cancer were clinically analysed and about 610,000 people were killed. Colon cancer is a disease that originates in the colon as a result of mutation in the signalling pathway. The common mutated gene in the colon cancer is the APC gene. APC protein is produced by this APC gene. The APC protein is said to ba brake in building of the β catenin protein. The β catenin is accumulated to the high level and is translocated to the nucleus. Then

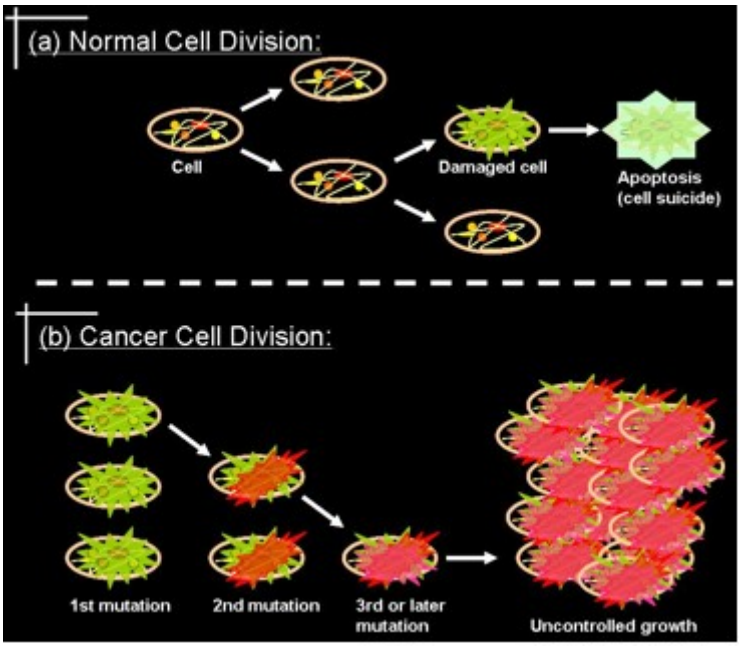
it binds to the DNA an it activates the gene transcription. When these are overexpressed it leads to cancer. APC is said to be mutated in every colon cancer. The apoptotic cancer which are mainly nuetralised in colon cancer are TGF-β and DCC. TGF-β consists of a disabled mutation in a minimum of half colon cancer. DCC usually has a removal of the chromosome in colon cancer. Some genes called as the oncogenes are overexpressed in the case of colon cancer. For example, the genes which has the protein KRAS,RAF and PI3 which usually help the cells to divide with the help of the growth factors. That will attain mutation by the over activation of the cell proliferation. (BN Ames; 1993) The spread of cancer from one part of the body to another is called metastasis. Cancer is not just one disease but many diseases there are more different types of cancer. Most cancers are named for the organ or type of cell in which they start for example cancer that begins in the colon is called colon cancer, cancer that begins in lung is called lung cancer, cancer that begin in the breast called breast cancer.[Rang et. al 2007]

Cancer types can be grouped into broader categories. The main categories of cancer include:

- **Carcinoma** – cancer that begins in the skin or in tissues that line / cover internal organs.
- **Sarcoma** – cancer that begins in bone, cartilage, fat, muscle, blood vessels, other connective tissue.
- **Leukemia**- cancer that begins in blood forming tissues such as the bone marrow and causes large number of abnormal blood cells to be produced and enter the blood.
- **Lymphoma and myeloma**: cancer that begin in the cells of immune system.
- **CNS cancers:** cancer that begin in the tissue of brain and spinal cord.

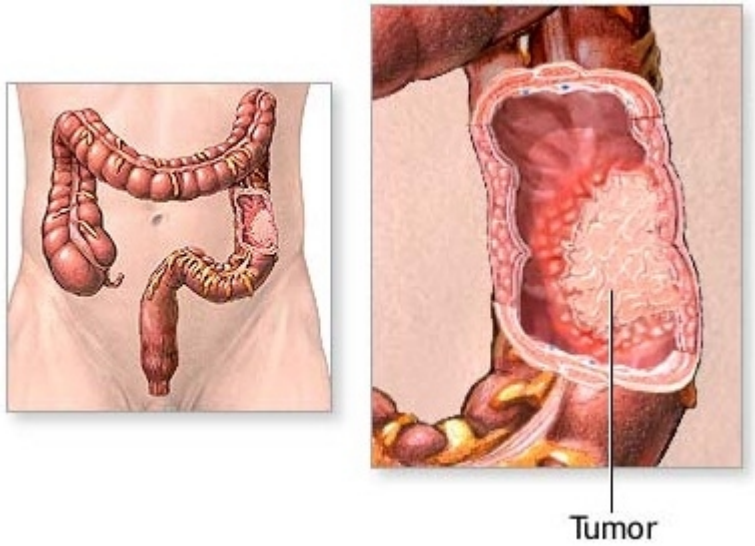
1.b. Etiology:

The appearance of these abnormal characteristics reflects altered patterns of gene expression in the cancer cells, resulting from genetic mutations.Cancer is a disease characterised by uncontrolled multiplication and spread of abnormal forms of the body's own cells. The neoplastic transformation include progression from pre cancerous lesion (typically more amenable to treatment) to malignant tumours (typically resistant to treatment strategies), caused by combination of both internal and external factors. For example i) exposure to external environmental factors such as radiation (UV, gamma and X-ray) damages cellular DNA and can lead to skin cancer ii) infectious micro organisms such as helicobacter pylori can cause gastric cancer; viruses such as papilloma virus causes cervical and hepatitis B causes liver cancer; iii) carcinogenic agents generated either endogenously through metabolic activities and smoking can contribute carcinogenic process.



In addition physiological oxidant anti oxidant is disrupted and more oxidants are produced, a cell or organism is said to be in oxidative stress. Ex: over production of oxygen free radicals and deficiency of (or) over helming anti oxidant defense or repair mechanisms can lead to O.S .under these

conditions ROS intermediate can damage DNA, which in turn can disrupt normal transcription and replication and induce mutations. Such oxidative damage to macro molecules can induce inflammatory signaling which in turn leads to cancer development. [Addanki.P Kumar et al, 2011].



COLON CANCER

1.c. CAUSES:

Colon cancer are never transmitable. The factors which is responsible of getting colon cancer are:

- People who are more than 60 years of age
- Involvement of processed meat in their diet
- Already having other cancers
- The person who has already developed colorectal tumours
- People having ulcers
- Having colon cancer in other members of the family
- Those who have breast cancer

Smoking cigarette and consumption of alcohol result in colon cancer

1.d. SYMPTOMS:

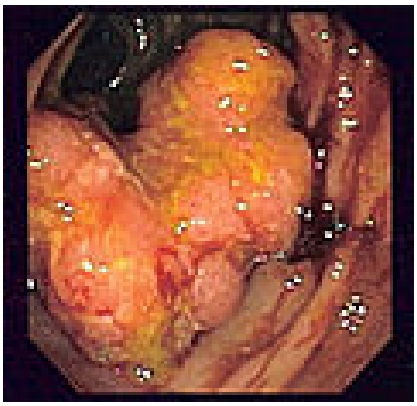
Colon cancer usually don’t show any symptoms. Some of the natural symptoms are:

- Pain in the abdomen
- Appearance of the blood in the stool
- Constipation and diarrhea
- Sudden weight loss.

1.e.SIGNS AND TESTS:

Colon cancer can be found out before any symptoms are developed. The doctors when press on the stomach they can feel the lump in the abdomen. Usually screening test are done for the colon cancer.

- Colonscopy
- Sigmoidscopy
- Blood test
- Liver test
- CT or MRI scan of the abdomen
- PET scans



ENDOSCOPIC IMAGE OF COLON CANCER IDENTIFIED BY COLONOSCOPY

1.f. The different stages of colon cancer:

Stage 0: Tumours in the innermost layer of the intestine.

Stage 1 Tumour in the inner layer of the colon.

Stage 2: Tumour spread in the muscles

Stage 3: Tumours extend to the lymph nodes

Stage 4: Tumours extend to other organs.

1.g. TREATMENT:

Treatment for the colon cancer depends on the different stages of cancer. The treatments consists of:

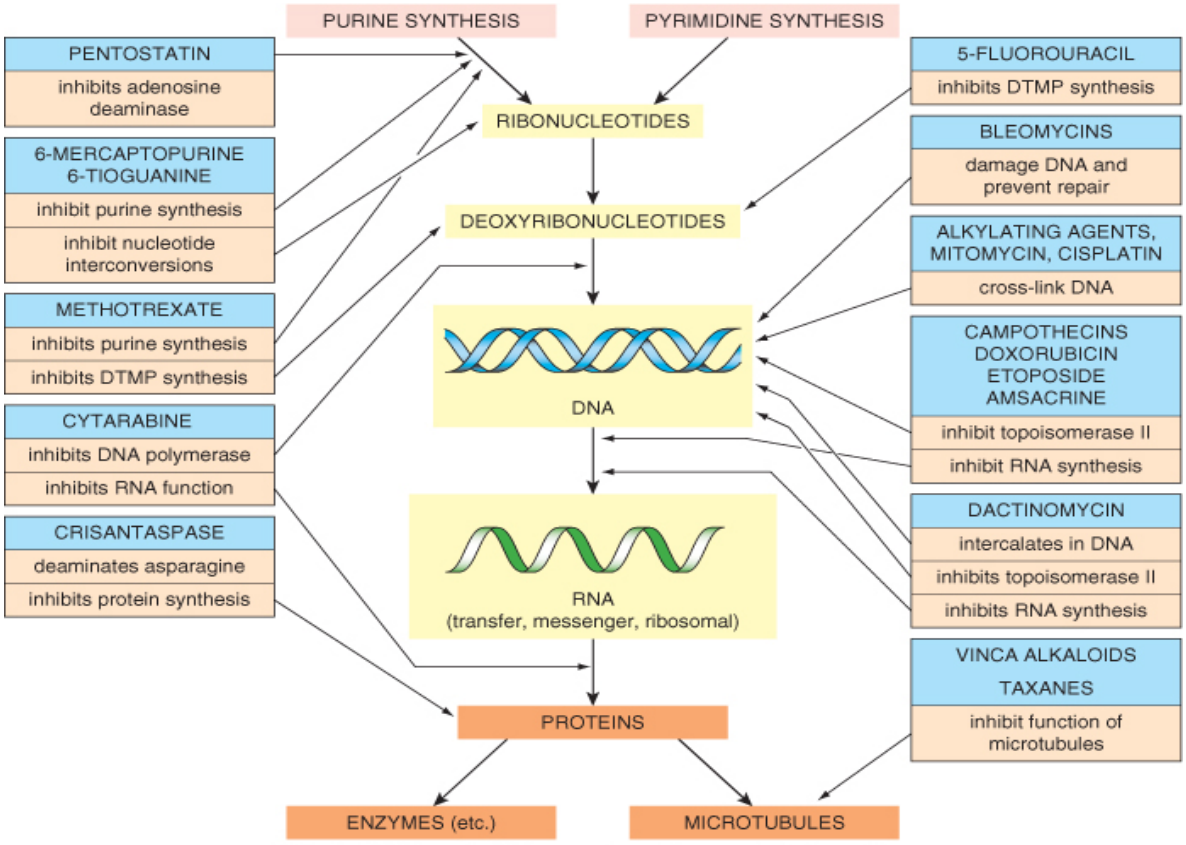
1.Surgery

2.Chemotherapy

3.Radiation

1.h. DRUGS USED IN CHEMOTHERAPY:

The main anti cancer drugs can divided into the following general categories:



i) **Principles of chemotherapy**:

1. Most common route of administration is Orally, Vein and Muscle. The othermethods are used to increase the concentration of drug at the tumor site

2. The patient's lab data needs to be checked before the onset of chemotherapy (eg) Blood cells count, Renal and Liver function.

3. Antinausea drugs and increased fluid intake is advised to avoid the side effect of anticancer drugs

ii) Combination chemotherapy:

Some of the anticancer drugs work better than alone.So two or more drugs are often given at the same time. This is called as combination chemotherapy. If the anticancer drug is used too frequently that will develop resistant to the drug. Sometimes the cancerous cells become resistant since the enzyme required to repair the damage DNA has increased. The one way to counter this problem is combination chemotherapy.

iii) Advantages:

1. Lower the concentration of the drug being administered and the side effects are decreased.

2. The drug selected in such a way that it can affect the cell cycle at different stages. Hence the DNA would not get repaired (Sunali Mehta et al).

iv) Categories of chemotherapeutic drugs:

In general, chemotherapy agents can be divided into three main categories based on their mechanism of action.

1. Stop the synthesis of Pre DNA molecule binding blocks:

These agents work in a number of different ways. DNA building blocks are folic acid, heterocyclic bases, and nucleotides these are made naturally within cells. All these agents are work to block some step in the formation of nucleotides or deoxyribonucleotides(necessary for making DNA).When those steps are blocked the nucleotides which are the building blocks of DNA and RNA can not be synthesized. So the cells can not replicate because they can not make DNA without the nucleotides. Examples of drugs in this class include 1) Methotrexate ,2) Fluorouracil, 3) Hydroxyurea, and 4) Mercaptopurine.

2. Directly damage the DNA in the Nucleus of the cell:

These agents chemically damage DNA or RNA. These disrupt replication of the DNA and either totally halt replication or cause the manufacture the new DNA or RNA does not code for anything. Examples of drugs in this class include Cisplatin and Antibiotics -Daunorubicin, Doxorubicin and Etoposide.

3. Effect the synthesis or breakdown of the mitotic spindles:

Mitotic spindles serve as a molecular railroads with "North and South Poles" in the cell when a cell starts to divide itself into two new cells. These spindles are most important because they help to split the newly copied DNA such that this copy goes to each of the two new cells during cell division. These drugs disrupt the formation of this spindle and therefore interrupt cell division. Examples of drugs in this class of miotic disrupters are: Vinblastine, Vincristine (Charles E Ophardt et al).

v) Side effects of anticancer drugs:

1. The healthy cells that are affected the most are under go rapid turnover (eg) Bone marrow cells, skin, hair etc. The other side effects are

2. Nausea

3. Vomiting

4. Hair loss

5. Disturbance in the GI Tract

6. Neutropenia (Low WBC count)

7. Thrombocytopenia (Low platelet count)

8. Mouth ulcers etc

SYMPTOMS:

1. Local colon cancer symptoms:

2. Local colon cancer usually effects our daily habits.

3. Systemic colon cancer symptoms:

4. System colon cancer affects our full body such as;

5. Loss of weight

6. Tired feling

7. Vomiting

8. The count of RBC decreases

9. Jaundice

RISK FACTORS:

Colon cancer is considered to be the most common cancer, about 72% of the cancer take place in the colon and 28% takes place in the rectum. The risk of the cancer in the colon increases with the age. It usually occurs in people who are more than 50. Men have more risk than the females.

FAMILY HISTORY OF COLON CANCER:

Colon cancer usually occurs in people who has a family history of the disease in patients with small percentage of the colon cancer usually has abnormality in the gene which result in the disease. The syndrome of mutation of genes consists of familial adenomatous polyposis and hereditary non-polyposis cancer.

FAMILIAL ADEMATOUS POLYPOSIS:

This is caused by the mutation in adenomatous polyposis coli gene whose main function is to control the growth of tumour. This also leads to formation of other types cancers which includes stomach, thyroid,liver,small intestine etc.

HEREDITARY NON POLYPOSIS COLON CANCER:

50-80% of people with abnormality in gene gets colon cancer at the age of 45. This disease mainly occurs by the mutation of MCH1, MSH2,MSHC genes. People who has this disese are subjected to other types of cancers which include uterus, ovarian cancers.

LIFE STYLE FACTORS:

Colon cancer risk is found to be more high in industrial nations. The mainly increased factor of the colon cancer like sedentary, smoking and excess weight.

Dietary factors:

The meat which is red and processed have a high risk of colon cancer. Friuits and vegetable consumption reduces the risk of colon cancer. Recent studies shows that the consumption of folic acid do not reduce the risk of cancer cancer instead it increases the formation of polyp.

Alcohol and smoking:

Alcohol and smoking increase the risk of colon cancer.

Obesity:

Obesity leads to the risk of colon cancer, especially in a case of men.

Physical inactivity:

An inactive lifestyle leads to the development of colon cancer. Regular exercise reduces the risk of colon cancer. (B N Ames; 1991)

BACKGROUND OF THE DISEASE COLON CANCER:

Colon cancer is considered to be the most common cancer found in both male and female and it is said to be the second highest for causing death in USA. This risk of this disease increases with age. This is a disease which is mainly seen in older population.

Colon cancer is a disease which is said to be very challenging in order to manage both economically as well as clinically. Economically the colon cancer is said to be costly disease and clinically colon cancer has more than one therapy for treatment. The diagnosis or the treatment of the disease is largely dependent an the various stages of the disease like tumour size lymph node etc. In this disease mainly five stages can be seen. In each stage many clinical factors can be determined such as the presence or absence of molecular markers, the number of lymph nodes present etc.(JH Weisburger 199

Colon cancer in different stages are:

In stage 1 cancer is present in the walls of the colon and can be cured bi surgery.

In stage 2 the partial spreading of the cancer.

In stage 3 it needs surgery and chemotherapy for six months.

In stage 4 the patient needs a surgery of the colon as well as the other parts of the body which are affected by colon cancer.(LR Howard; 2003)

BLUEBERRY:

Blueberry is a fruit or a shrub which usually grow in clusters. There are different kinds of blueberry which usually have a large source of antioxidants. Blueberries are used in the preparation of jams, jellies. Regular consumption of blueberry results in the increase of the benefits of human health. Blueberries consists of many phytochemicals which has many biological properties such as:

- Antioxidant
- Anticancer
- Anti neurodegenerative
- Anti-inflammatory.

ANTIOXIDANT EFFECT:

Blueberry is considered to be an influential antioxidant. The main fuction of the blueberries is that it gives protection to cell from damaging which is mainly caused by oxygen free radicals. Antioxidants play an important role in the nueteralisation of free radicals. The free radicals are said to be the unsatable molecules which plays a role in the development of a number of disease and conditions which consists of cancer , cardiovascular disease etc. Fruits and vegetables are said to be the important source of antioxidants among which the blueberries is said to have the highest antioxidant activity. (LR Prior 2006)

ANTICANCER EFFECT:

In a study made in the university of Georgia in 2005 showed that the blueberry extract has the capacity to stop the proliferation of the cells in colon cancer cell lines. Cancer cells can grow and attack the tissues by the secretion of the enzyme. Many studies showed that the active component present in the blueberry stops the activity of the enzymes which play a role in the spreading of the disease to different organs and tissues.

Blueberries contain many active components of which anthocyanin ,pterostilbene, polyphenols are important which act as an antioxidant and helps to inhibit the cancer cell formation.(M P Kahkonen 2001)

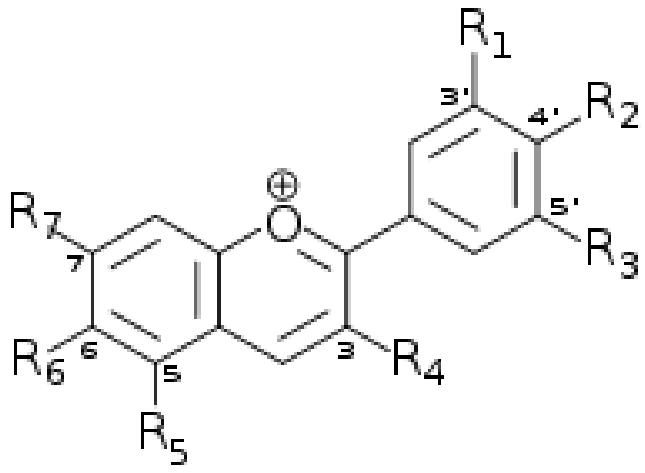
ANTHOCYANIN:

Anthocyanins which are present in blueberries are the derievatives of cyanidindelphinidin ,malvidin , pentunidin and peonidin. Anthocyanins is said to be the active component present in blueberry which plays important role in the prevention of colon cancer. (M P Kahkonen 2001)

ANTICANCER PROPERTIES OF ANTHOCYANIN:

The structure of antocyanin is responsible for the antioxidant activity. The antioxidant property of the anthocyanin has been experimented using various cell culture lines like colon , breast , liver etc. The antioxidant activity of anthocyanins is mainly due the presence of the hydroxyl group. Pure anthocyanins rich extract from the blueberries shows anti poliferative activity. The proliferation of the cells were stopped by the anthocyanins which blocks the different stages of cell cycle. Several investigation were made in which the antipoliferative activity of the anthocyanins of the cancer cells is compared with normal cells. The result showed that the anthocyanin inhibit the growth of cancer cells which shows small effect or rather no effect on the growth of normal cells. Anthocyanins stop the growth of malignant cell and apoptosis are stimulated. Many studies shows that the uptake of anthocyanins in humans and they reach a level between 10^{-8} and 10^{-7} M in human blood. Anthocyanins show anticarcinogenic activity which play role against many cancer cell types. Many studies conducted shows that the absorption of anthocyanins plays an important role in the chemoprevention of cancer in humans especially in tissues rather than the gastrointestinal tract.

Anthocyanins are the ones which occur in fruits and vegetables which has glucose, xylose etc. The anthocyanins always has a positive charge in acidic solution. They are soluble in water. The sugar components which is present in anthocyanins are mixed with C3 hydroxyl group. Numerous anthocyanins vary in their skeleton and position in which the glycosides are attached to the skeleton.(WC WILLET 1995)



POLYPHENOLS:

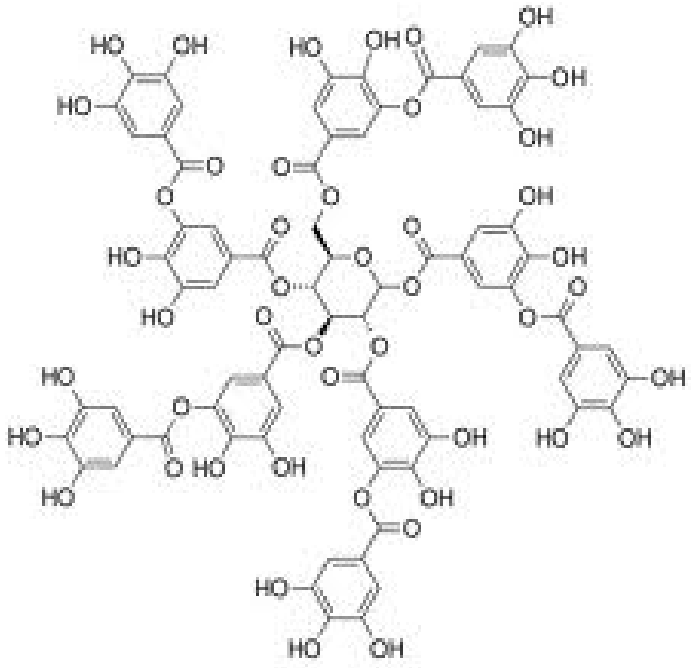
Polyphenols is said to be a kind of natural chemical present in plants. They are the antioxidants which prevent the damage of cells in the body. Polyphenols behave as antioxidants in humans. This helps in the eradication of free radicals from human body. Free radicals are unstable molecules that result in cell damaging. This is called oxidative stress. So the main roles of polyphenols is to reduce the oxidative stress by soothing down the free radical molecules.

Polyphenols are said to be the most profuse antioxidant present in the diet. The dietary intake is much higher than that of other dietary antioxidant. The main dietary sources are fruits such as blueberry, tea , coffee, red wine etc. Vegetables , cereals ,

Chocolates play important role in the intake of polyphenol.

Although they are widely distributed in fruits, the benefits of health of polyphenols were identified by the nutritionist very recently. Research studies on polyphenols, flavanoids the antioxidant property and the prevention of the disease mainly began after 1995. There was a delay on the study of the antioxidant property is mainly because of its complicated chemical structure. The main difficulty in explicating the health effects of polyphenols is the large amount of phenolin compounds present in food which leads to different biological activity. Polyphenols are widely conjugated in the body. The relationship between the antioxidant and the disease are not completely understood and are gone through intensievely. Polyphenols and other antioxidants acts as a safeguard against many oxidative injury. As antioxidants cells helps to improve the survival of the cell and also stops tumour growth. Cells usually react to the polyphenols mainly by the direct connections with the

enzymes which plays an important role in the signal transduction which helps in the adaptation of the redox position of the cell and result in the generation of the cell and activates a number of redox reactions. the best example is the communication of isoflavones with oestrogen receptors and they effect on the function of endocrines. A thorough description of the molecular events that are the fundamentals of the different biological effect is important to understand the various risk of the disease and its progression.(LR Howard 2003)



CHEMICAL STRUCTURE OF POLYPHENOLS

CURRENT UK STATISTICS:

The incidence of colon cancer depends upon age. It mainly occurs in people with age more than 60. Till the age of 50 the rate of colon cancer is similar but after the age of 50 the rate of colon cancer is said to be high in females than in males. The overall ratio for male and female is 11:10.

The most important advantage is that between 5% and 9% relative survival for the most wealthy patients in comparison with the poor groups. If this difference between the rich and poor is removed then about 3000 deaths would be avoided within the last five years. The rate of colon cnacer across Europe shows certain differences. It has been stated that the poor survival in UK

compared with the rest of western Europe is because of the delay for treatement. The exsistence of differences in survival depending on place and time recommend that there are ways in which diagnosis could be removed in coutries such as UK.The present evidence regarding the protective effect of polyphenols has made new expectation for the health improvement and the development of products rich in polyphenol. It is not possible to estimate the individual benefits that increase the intake of polyphenol. (S Bardona 2002)

REVIEW OF LITERATURE

CorineDjadjoDjipa et.al (2000) examined the Acetone and aqueous extracts of the bark of *S. jambos* were tested for antimicrobial activity invitro by the agar dilution method. Both extracts showed some activity against the tested micro-organisms. They proved to be particularly effective on *Staphylococcus aureus*, *Yersinia enterocolitica*and coagulase negative staphylococci among which *Staphylococcus hominis*, *Staphylococcus cohnii*and *Staphylococcuswarneri*. These properties seem to be related to the high tannin content of *S. jambos*extracts (77 and 83% for the aqueous and acetone extracts).

T. Vithya et.al (2012) reported on the free radical scavenging activity of *sophoraineterupta* by lipid peroxidation and nitric oxide method. It was evaluated by using the Nitric oxide scavenging method and Lipid peroxidation method. In the earlier mentioned method the IC50 value of the extract was found to be>1000 which was compared with the standard Rutin which showed an IC50 value of 65.44 and in the later method the IC50 value of 115.00 which was compared with the standard BHA which showed an IC50 value of 230.

Francois Denizot and Rita Lang et al **(1986**) reported on a convenient way to estimate the number of viable cells growing in microtitre tray wells is to use a colorimetric assay and an automatic microplate scanning spectrophotometer

Ni Zheng et al(2011)reported on the antioxidant activity of total flavonoids extract from *Syzygiumjambos*seeds (TFSJ). Response surface methodology was employed to optimize the main extraction conditions including extraction time, ethanol concentration and solid-liquid ratio. The effects of TFSJ on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, 2,2-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging and hydroxyl radical (HO•) scavenging were studied. Under the conditions of extraction time 32.27 min, solvent concentration 52.01% and solid-liquid ratio to be 27.32:1, TFSJ possesses considerable amounts of flavonoids of 11.4330

mg/g rutin equivalent of extract. The effect of this extract in scavenging activity of ABTS and hydroxyl radical activity was better than that of rutin. However, the effect of TFSJ in free radical scavenging of DPPH was not as good as that of rutin.

J. Agric. Food Chem1988.46 et al Antioxidant activity of methanolic extract evaluated according to the β-carotene bleaching method expressed as AOX (Δ log A_{470}/min), AA (percent inhibition relative to control), ORR (oxidation rate ratio), and AAC (antioxidant activity coefficient) ranged from 0.05, 53.7, 0.009, and 51.7 to 0.26, 99.1, 0.46, and 969.3, respectively

*LuisaDuqu2013etal*2,4-Dihydroxy-9-phenyl-1*H*-phenalen-1-one(4hydroxyanigorufone, 1), a compound isolated from *Anigozanthos flavidus* and *Monochoria elata*, displayed a high radical scavenging capacity in the ORAC assay. A systematic approach was adopted in order to explore ...

Soumya maithy 2013 Evaluation of Antioxidant Activity and Characterization of Phenolic Constituents of Phyllanthus amarus Root The root extracts showed higher DPPH, hydroxyl, superoxide, and nitric oxide radical scavenging and reducing power activity. Among all the samples, the ethyl acetate-soluble fraction demonstrated highest radical scavenging activity and total phenolics content. Twenty-eight different phenolic compounds were identified by LCMS/MS analysis of the ethyl acetate-soluble fraction.

Debora villario al el 2009 774 When blueberries and milk were ingested there was no increase in plasma antioxidant capacity. There was a reduction in the peak plasma concentrations of caffeic and ferulic acid (− 49.7%, $p < 0.001$, and − 19.8%, $p < 0.05$, respectively) as well as the overall absorption (AUC) of caffeic acid ($p < 0.001$). The ingestion of blueberries in association with milk, thus, impairs the in vivo antioxidant properties of blueberries and reduces the absorption of caffeic acid. The antioxidant properties of dietary phenolics are believed to be reduced in vivo because of their affinity for proteins. In this study we assessed the bioavailability of phenolics and the in vivo plasma antioxidant

capacity after the consumption of blueberries (*Vaccinium corymbosum L.*) with and without milk. In a crossover design, 11 healthy human volunteers consumed either (a) 200 g of blueberries plus 200 ml of water or (b) 200 g of blueberries plus 200 ml of whole milk

Smithe et al Fruit extracts of four *Vaccinium* species (lowbush blueberry, bilberry, cranberry, and lingonberry) were screened for anticarcinogenic compounds by a combination of fractionation and *in vitro* testing of their ability to induce the Phase II xenobiotic detoxification enzyme quinone reductase (QR) and to inhibit the induction of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine synthesis, by the tumor promoter phorbol 12-myristate 13-acetate (TPA). The crude extracts, anthocyanin and proanthocyanidin fractions were not highly active in QR induction whereas the ethyl acetate extracts were active QR inducers

Michael D Matchett 2005,85 et al Regulation of the matrix metalloproteinases (MMPs), the major mediators of extracellular matrix (ECM) degradation, is crucial to regulate ECM proteolysis, which is important in metastasis. This study examined the effects of 3 flavonoid-enriched fractions (a crude fraction, an anthocyanin-enriched fraction, and a proanthocyanidin-enriched fraction), which were prepared from lowbush blueberries (*Vaccinium angustifolium*), on MMP activity in DU145 human prostate cancer cells in vitro. Using gelatin gel electrophoresis, MMP activity was evaluated from cells after 24-hr exposure to blueberry fractions. All fractions elicited an ability to decrease the activity of MMP-2 and MMP-9. Of the fractions tested, the proanthocyanidin-enriched fraction was found to be the most effective at inhibiting MMP activity in these cells. No induction of either necrotic or apoptotic cell death was noted in these cells in response to treatment with the blueberry fractions

Henry S. Scheuller et al 2006 The berry extracts were also evaluated for their ability to stimulate apoptosis of the COX-2 expressing colon cancer cell line, HT-29. Black raspberry and strawberry extracts showed the most significant pro-apoptotic effects against this cell line. The data provided by the current study and from other laboratories warrants further investigation into the chemopreventive and chemotherapeutic effects of berries using in vivo models.

Lynn S. Adams et al 2006 The major classes of berry phenolics were anthocyanins, flavonols, flavanols, ellagitannins, gallotannins, proanthocyanidins, and phenolic acids. The berry extracts were evaluated for

their ability to inhibit the growth of human oral (KB, CAL-27), breast (MCF-7), colon (HT-29, HCT116), and prostate (LNCaP) tumor cell lines at concentrations ranging from 25 to 200 μg/mL. With increasing concentration of berry extract, increasing inhibition of cell proliferation in all of the cell lines were observed, with different degrees of potency between cell lines.

. SCOPE, OBJECTIVE AND PLAN OF WORK

The search for anti-cancer agent in modern times was marked by the introduction of cancer for the treatment of colon cancer due to kidney infection prostate, nephritis and enuresis. herbal medicines are in line with nature, no hazardous reaction of late the interest in plant products rose all over the world due to the belief they many herbal medicine are known to be free from side effect although this statement can be debatable further more the fact that the discovery of a new synthetic drug is the time consuming expensive affair

Blue berry belonging to the family Myrtaceae, are traditionally used in cancer ,ulcer and in americans of blue berry fruit are given to ulcer anticancer Blueberry Phenolics and their antioxidant activity; J Agric Food Chem.However the antioxidant efficacy of the fruit extract has not been studied. Hence the present study was carried out to evaluate the antioxidant potential of the acetone extract of Vacciniumpallidum

In vitro antioxidant, anticancer studies were done as the use of animals in pharmacological screening has been restricted on ethical basis nowadays. So we done in cell lines and reduce the animal experimental work

OBJECTIVE OF WORK:

The present study was designed to assess the *Invitro*anti oxidant, and anti cancer activity of acetone extract fruit of Vacciniumpallidum.

PLAN OF WORK

- Collection and identification of plant
- Extraction
- Phytochemical analysis
- Evaluation of anti oxidant activity:
  - ❖ DPPH assay
  - ❖ Nitric oxide scavenging assay
  - ❖ Lipid peroxidation assay
  - ❖ Total antioxidant capacity.
- Evaluation of anti cancer activity:
  - ❖ HT-29 (Human colorectal cancer) cell line
  - ❖ HCT116 (Human colorectal cancer) cell lines
  - ❖ HC115 (Human colorectal cancer) cell lines

PLANT PROFILE

FRUIT AND LEAVES of BLUE BERRY OF ***Vacciniumpallidum***

Scientific Classification

Kingdom	:	**plantae**
Division	:	**tracheobionta**
Class	:	**magnoliophyta**
Order	:	**magnoliopsida**
Sub Class	:	**dilleniidae**
Order	:	**ericales**
Family	:	**ericaceae**
Genus	:	**vaccinium**

ScientificnameVacciniumpallidum

Distribution : Throughout north America and india in Kashmir**.**

Habitat : The plants, naturally growing at the edge of bogs above the edge of high water marks in a thick mat of high acid mulch. Often growing in sandy soils above hardpan, blueberries require ample moisture but are not bog plants. For success with blueberries, try to duplicate a native blueberry's natural habitat in the home garden by supplying well-drained acid soil high in organic material and adequate water.

Botanical description.

The leaves can Beither deciduous or evergreen, ovate to lanceolate, and 1–8 cm (0.39–3.1 in) long and 0.5–3.5 cm (0.20–1.4 in) broad. The flowers are bell-shaped, white, pale pink or red, sometimes tinged greenish. The fruit is a berry 5–16millimeters (0.20–0.63 in) in diameter with a flared crown at the end; they are pale greenish at first, then reddish-purple, and finally dark blue when ripe. They have a sweet taste when mature, with variable acidity. Blueberry bushes typically bear fruit

in the middle of the growing season: fruiting times are affected by local conditions such as altitude and latitude, so the height of the crop can vary from May to August depending upon these conditions.

Composition of Blueberries

Blueberries were analyzed for procyanidins. Monomers, identified as (+)-catechin and (-)-epicatechinand a series of oligomers were detected. The oligomers consisted of epicatechin units singly-linked (B-type). The procyanidin fraction accounts for up to 32% of the total ORAC measured in blueberries. Investigated changes in blueberry anthocyanins and polyphenolics during processing into juice and concentrate. 32% of the anthocyanins were recovered in single-strength juice while flavonol, procyanidin and chlorogenic acid recoveries in juice were 35%, 43% and 53%, respectively. The proportion of polyphenolics remaining in the press-cake residue ranged from 1% to 18%. Anthocyanin profile changed with processing because of varying stability of individual pigments with malvidin glycosides being most stable and delphinidin glycosides the least.

Medicinal use

- Blue berry juice is a good natural remedy for disease of urinary tract.
- A natural medicine for eyes.
- A good solution for incipient diabetes
- A remedy to improve circulation.
- A effective solution against wond and ulcer.

MATERIALS AND METHODS

Materials:

a) Plantmaterial:Blueberry(Vacciniutillus)

b) Collection of plant material:

Authentified plant material was collected from Cancer Research centre,

2. Methods:

1. Extraction of plant material

The collected powdered materials (100g each) were extracted with 70%Actone in De-ionized Water in a Seven Speed Stainless steel Laboratory Blender Cooled Over Night .The Chilled Blue Berry Filter Through Five Layer Cheesecloth. Then Filter Centrifuge For 10 minutes the Supernated and poure in to pyrex baking dish and place under fume hood to allow for evaporation of acetonFijesh et al, 2010)

2. PRELIMINARY PHYTOCHEMICAL EVALUATION:

The Acetone extract obtained from Blueberry (Vacciniummyrtillus) were subjected to qualitative phytochemical investigation for identification of various constituents.

i. Test for Alkaloids:

Small amount of extract were stirred with a few ml of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloid reagents. Such as Mayer's, Dragendroff's, Wagner's and Hager's reagent.

a) Mayer's test (Potassium mercuric iodide):

To 1 ml of the filtrate few drops of Mayers reagent was added, Formation of cream colour precipitate indicates the presence of alkaloids.

b) Dragendroff's test (Potassium bismuth iodide):

To 1 ml of the filterate few drops of dragendroff's reagent was added formation orange- red, precipitate indicates the presence of alkaloids.

c) Wagner's, test (Iodide solution):

To 1 ml of the filterate add few drops of Wagner's reagent. Formation of brown precipitate indicates the presence of alkaloids.

d) Hager's test (Picric acid):

To 1 ml of the filterate add few drops of Hager's reagent. Formation of orange yellow colour indicates the presence of alkaloids.

ii. Test for Carbohydrates:

The test solution was prepared by dissolving the test extract with water. Then it was hydrolyzed with 1 volume of 2N HCl and subjected to following chemical tests.

a) Molisch's test: Test solution with few drops of Molisch's reagent an 2 ml of conc.

HSOadded slowly from the sides of the test tube. A purple ring atthe junction of twoliquids indicates the presence of Carbohydrates.

b) Barfoed's test:

Test solution treated with Barfoed's reagent and after boiling on a water bath, it showed brick red colour precipitate of indicates the presence of Carbohydrates

c) Benedict's test:

Mixed equal volume of Test solution treated with Benedict's reagent and after boiling on water bath, it show reddish brown precipitate of indicates the presence of Carbohydrates.

d) Fehling's test:

The test solution when heated with equal volume of Fehling's A and B solutions, give orange red precipitate, indicates the presence of Carbohydrates

iii. Test for Flavonoids:

To a small amount of extract added equal volume of 2M HCl and heated in a test tube for 30 to 40 min at 100°C. The cooled extract was filtered, and extracted with ethyl acetate. The ethyl acetate extract was concentrated to dryness, and used to test for flavonoids.

a) Lead acetate test:

To small quantity of residue, added lead acetate soluction. A yellow coloured

precipitates, indicates the presence of flavonoids.

b) Sodium hydroxide test:

Addition of increasing amount of Sodium hydroxide to the residue showed yellow coloration, which decolouration after the addition of acid. This indicates the presence of flavonoids.

iv. Test for Fixed Oils:

a) Spot test:

A small quantity of extract was pressed between two filter papers. Stain in aqueous extract indicates the presence of fixed oil. b) Saponification test:

Few drops of 0.5 N alcoholic potassium hydroxide was added to extract along with a few drops of phenolphthalein. The mixture was heated on water bath for one hour. Formation of soap indicates the presence of fixed oil.

v) Test for Phytosterols:

Small quantity of extract was dissolved in 5ml of chloroform separately. Then the chloroform layer was subjected to salkowaski and Libermann- Bur chards test.

a) Salkowaski test

A few drops of concentrated sulphuric acid were added to chloroform solution. The lower layer of solution turned brownish red colour indicates the presence of phytosterols.

b) Liebermann- Burchard test:

A few drops of acetic anhydride were added to chloroform solution. Then to this concentrated sulphuric acid was added through the sides of the test tube. A brown ring was formed at the junction and the upper layer turned green colour, indicates the presence of phytosterols.

vi. Test for Glycoside:

Small quantity of the extract was hydrolyzed with hydrochloric acid for two hours on a water bath and the hydrolysis was subjected to Legal's and Borntraggers'stest for detecting the presence of different glycosides.

a) Legal's test:

To the hydrolysis extract of 1ml of pyridine and few drops of sodium nitroprusside solution were added, and then it was made alkaline with sodium hydroxide. Appearance of pink to yellow colour, indicates the presence of glycosides.

b) Brontragger's test:

Hydrolyses extract was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added.Appearance of pink colour indicates the presence of glycoside.

vii. Test for Proteins:

Small quantities of the extracts were dissolved in few ml of water and they were subjected to, millons test, biurets test.

a) Million's test:

Test solution was treated with Million's reagent and heated on a water bath. The proteins were stain, the protein were indicates the red colour.

b) Biuret test:

Test solution was treated with 40% sodium hydroxide and dilutes copper sulphate solution, the protein were stain indicates the blue colour.

viii. Test for Tannins:

a) 5% FeCl3 solution:

Take 2-3 ml of alcoholic or aqueous extract than added few drops of 5%FeCl3 reagents, deep blue-black colour indicates the presence of tannins.

b) Lead acetate solution:

Take 2-3 ml of alcoholic or aqueous extract than added few drops of Lead acetate solution, White precipitate indicates the presence of tannins.

c) Bromine water:

Take 2-3 ml of alcoholic or aqueous extract than added few drops of Bromine water, Discoloration of bromine water indicates the presence of tannins.

ix. Test for Triterpenoids:

The test extract solution was prepared by dissolving extract in the chloroform

a) Salkowaski test:

Few drops of concentrated sulphuric acid were added to the test solution, shaken and golden yellow layer was obtained, indicates the presence of triterpenoids.

b) Libermann - Burchard Test:

To the test solution of the extract, few drops of acetic anhydride were added and mixed well. Then 1ml of concentrated sulphuric acid added from the sides of the test tube, a red colour was produced in the lower layer, indicates the presence of triterpenoids

x. Test for Amino acids:

Prepared test solution by dissolving the extract in water.

a) Ninhydrin test:

Test solution treated with Ninhydrin reagent gives blue colour was obtained, indicates the presence of amino acid.

I) In vitro of anti-oxidant activity

Materials and Methods

1. DPPH radical scavenging Assay: The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.

Reagents:

2,2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 μM)*:* 22 mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 μM DPPH solution.

a) Preparation of test solution: 21mg each of the extracts was dissolved in distilled DMSO separately to obtain solutions of 21 mg/ml concentrations. Each of these solutions was serially diluted separately to obtain lower concentrations

Preparation of standard solutions: 10 mg each of ascorbic acid and rutin were weighed separately and dissolved in 0.95 ml of Dimethyl sulfoxide (DMSO) to get 10.5 mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

Procedure*:* The assay was carried out in a 96 well microtitre plate. To 200 μl of DP PH solution, 10 μl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812 μg/ml. The plates were incubated at 37^{o} C for 30 min and the absorbance of each solution was measured at 490 nm, using a micro plate reader.

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control-(absorbance of test–absorbance blank)}}{\text{Absorbance of control.}} \times 100$$

2. Scavenging of Nitric Oxide radical: Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified GriessIlosvay reaction. In the present investigation, GriessIlosvay reagent is modified by using Naphthyl ethylene DiamineDihydro chloride (0.1% w/v) instead of 1-napthylamine (5%). Nitriteions react with Griess reagent, which forms a purple azo dye. In presence of test components, likely to be scavengers, the amount of nitrites will decrease. The degree of decrease in the formation ofpurpleazo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540 nm (Garratt, 1964; Nenadis et al, 2004)**.**

Reagents:

Sodium nitroprusside solution: Weighed accurately 0.2998 g of sodium nitroprusside and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask (10 mM).

Naphthyl Ethylene DiamineDihydrochloride (NEDD, 0.1%):

Weighed accurately 0.1 g of NEDD and dissolved in 60 ml of 50% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask with distilled water.

Sulphanilic acid (0.33% w/v) reagent: Weighed accurately 0.33 g of sulphanilic acid and dissolved in 20% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask.

Preparation of test sample: The sample solution was prepared as described in DPPH assay.

Preparation of standard solutions:

Weighed accurately 10 mg of ascorbic acid and rutin and dissolved in 1 ml of DMSO separately. From these solutions, serial dilutions were made to obtain lower concentrations using DMSO.

Procedure*:*

The reaction mixture (6 ml) containing sodium nitroprusside (10 mm, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25^{o} C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink colouredchromophore was formed. The absorbance of these solutions was measured at 540 nm.

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control-(absorbance of test –absorbance blank)}}{\text{Absorbance of cAbsorbance of control}} \times 100$$

3. Lipid peroxidation inhibitory activity:(Huong*et al.,* 1998)

Lipid peroxidation can be initiated by ROS such as hydroxyl radicals by extracting a hydrogen atom from lipids and forming a conjugated lipid radical. This reacts rapidly with oxygen to form a lipid radical until the chain reaction is terminated. The lipid peroxidation adducts may induce the oxidation of biomolecules such as DNA, proteins and other lipids resulting in cellular damage.

a) Preparation of egg lecithin:

Separate the egg yolk and wash it with acetone until yellow colour disappears. The creamy white powder thus obtained is used for the procedure by dissolving in phosphate buffer PH 7.4 (3mg/ml).

b) Procedure:

The reaction mixture containing egg lecithin (1ml), ferric chloride (0.02ml), ascorbic acid (0.02ml) and extract or standard (0.1ml) in DMSO at various concentrations was kept for incubation for 1 hour at 37ºC. After incubation 2 ml of 15% TCA and 2ml of 0.37% TBA were added. Then the reaction mixture was boiled for 15 min, cooled, centrifuged and absorbance of the supernatant was measured at 532 nm.

4. Evaluation of Total Antioxidant capacity of the extract: The total antioxidant capacity was determined by phosphomolybdenum method and is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex which has the maximal absorption at 695 nm.

Preparation of test and standard solutions:

Weighed accurately 55 mg of each extracts and the standard, ascorbic acid and dissolved in 5 ml of DMSO. The lower dilutions were made serially with DMSO.

Procedure:

An aliquot of 0.1 ml of the sample solution containing a reducing species in DMSO was combined in an Eppendroffs tube with 1 ml of reagent solution (0.6 M Sulphuric acid, 28 mm sodium phosphate, and 4 mm ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90 min. The samples were cooled to room temperature, and the absorbance of each solution was measured at 695 nm. The total antioxidant capacity was expressed as mm equivalent of ascorbic acid (Mojca*et al.*, 2005).

IV)Evaluation of anti-cancer activity:

Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyltetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai.DimethylSulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Cell lines and Culture medium:

Hct116, HT115and HT-29, cell lineswas procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml)and amphotericin B (5 μg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions:

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

Principle: The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

Procedure:The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37^o C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37^o C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD test group}}{\text{Mean OD of control group}} \times 100 \right)$$

RESULTS

Percentage yield of fruit extract:

The yield obtained from the Aceton fruit extract of vacciniumpallidum (F) Blueberry. Was about 162 grams (13.5 %) from 1.2 kg leaves.

Identification of phytochemical analysis

The result obtained after preliminary phytochemical analysis of Aceton fruit extract of vacciniumpallidum (F) Blue berry (Table no:) showed the presence of

- ❖ Carbohydrates
- ❖ Saponins
- ❖ Alkaloids
- ❖ Flavonoids
- ❖ Phenolics
- ❖ Tannins
- ❖ Phyto Sterols
- ❖ Triterpinoids

And the Absence of

- ❖ Cardiac glycosides
- ❖ Fixed oils

1	❖ **Test for carbohydrates** Molisch's test	+
2	❖ **Test for Glycosides** Modified Bomtrager's test Keller- Killiani test	—
3	❖ **Test for Saponins** Foam test	+
4	❖ **Test for Alkaloids** Mayer's test Dragendrodroff's test	+
5	**Test for Flavonoids** Alkaline reagent test	+
6	❖ **Test for Phenolics and Tannins** Ferric chloride test Test for Tannins	+
7	❖ **Test for Phytosterols and Triterpenoids** Leiberman-Bucharat test Salkowaski test	+
8	❖ **Test for fixed oils and fats** Oily spot test	—

Table no : Phytochemical analysis Aceton fruit extract of vacciniumpallidum (F) Blue berry

+ indicate s presence: indicates absence -

i) Invitroantioxidant activity:

DPPH radical scavenging assay:

The results of the ***in vitro*** antioxidant activity of Acetone fruit extract of vacciniumpallidum (F) Blue berry INDPPH radical scavenging assay are given in (Table No: 1), The IC50 value of the standard (Rutin) has been adopted from the previous studies (ShrishailappaBadami et.al)

S.No	Concentration In µg/ml	Mean of test Absorbance	Avg-blank	% of inhibition	
1	**200**	**0.128±0.001 ****	**0.057±0.001**	**60.4**	
2	**100**	**0.135±0.004**	**0.064±0.008**	**54.18**	
3	**50**	**0.143±0.007**	**0.079±0.007**	**42.09**	
4	**25**	**0.158±0.01 ***	**0.093±0.02**	**38.18**	**IC 50 values 84±1.8**. *
5	**12.5**	**0.173±0.004**	**0.101±0.003**	**29.04**	
6	**6.15**	**0.188±0.002 ***	**0.108±0.008**	**22.21**	
7	**3.12**	**0.195±0.0003**	**0.121±0.0002**	**16.03**	
8	**Control**	**0.220±0.006**	**0.142±0.006**	**0.0**	
9	**Standard Rutin**	**-**	**-**	**-**	**IC 50: 46.50±1.76**

Table no 4 : DPPH assay of Aceton fruit extract of vacciniumpallidum (F) Blue berry

Values are the average of (n=3) and represented as mean ± standard deviation and probability value * P<=0.05 significant when IC50 values are compared with standard. .

Acetone fruit extract of vacciniumpallidum (F) Blue berry– DPPH radicalscavengingassay IC 50 Value:

The results of the ***in vitro*** antioxidant activity of Acetone fruit extract of vacciniumpallidum (F) Blue berry INDPPH radical scavenging assay are given in (fig no : 23), The IC50 value of the standard (Rutin) has been adopted from the previous studies (Shrishailappa Badami et.al)

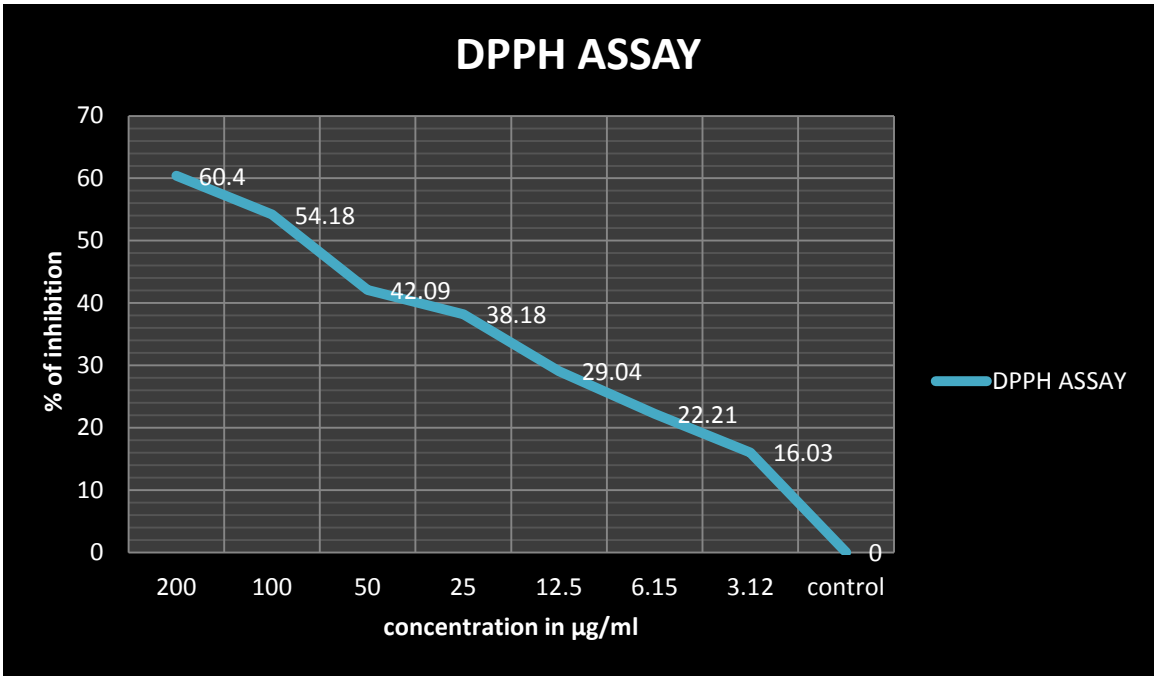


Fig no: 6 DPPH radical scavenging assay IC50 value

ii) Nitric oxide scavenging assay:

The results of the ***in vitro*** antioxidant activity of Acetone fruit extract of vacciniumpallidum (F) Blue berry IN Nitric oxide scavenging assay are given in (Table no : 2), The IC50 value of the standard (Rutin) has been adopted from the previous studies (P.Sivakumar et.al)

S. No	Concentration In µg/ml	Mean of test Absorbance	Avg-blank	% of inhibition	IC50 value
1	**500**	**0.165±0.001**	**0.038±0.001**	**71.8**	**66± 1.6***
2	**250**	**0.167±0.0005**	**0.047±0.0005**	**62.1**	
3	**125**	**0.172±0.002 ***	**0.054±0.002**	**56.30**	
4	**61.5**	**0.173±0.002**	**0.055±0.002**	**48.50**	
5	**31.2**	**0.175±0.0017**	**0.059±0.0017**	**34.8**	
6	**15.6**	**0.186±0.002**	**0.069±0.002**	**26.1**	
7	**7.8**	**0.196±0.0015**	**0.087±0.0015**	**17.07**	
8	**Control**	**0.214**	**0.12**	**0**	
9	**Standard Rutin**	**-**	**-**	**-**	**60.44**

Table no: 5 Nitric oxide radical scavenging activity of Aceton fruit extract of vacciniumpallidum (F) Blue berry.

Values are the average of (n=3) and represented as mean ± standard deviation and probability Values * P<=0.05; significant when IC50 values are compared with standard values.

Nitric oxide scavenging assay:

The results of the ***in vitro*** antioxidant activity of Acetone fruit extract of vacciniumpallidum (F) Blue berry innitric oxide scavenging assay are given in (fig no : 24), The IC50 value of the standard (Rutin) has been adopted from the previous studies .(P.Sivakumar et.al)

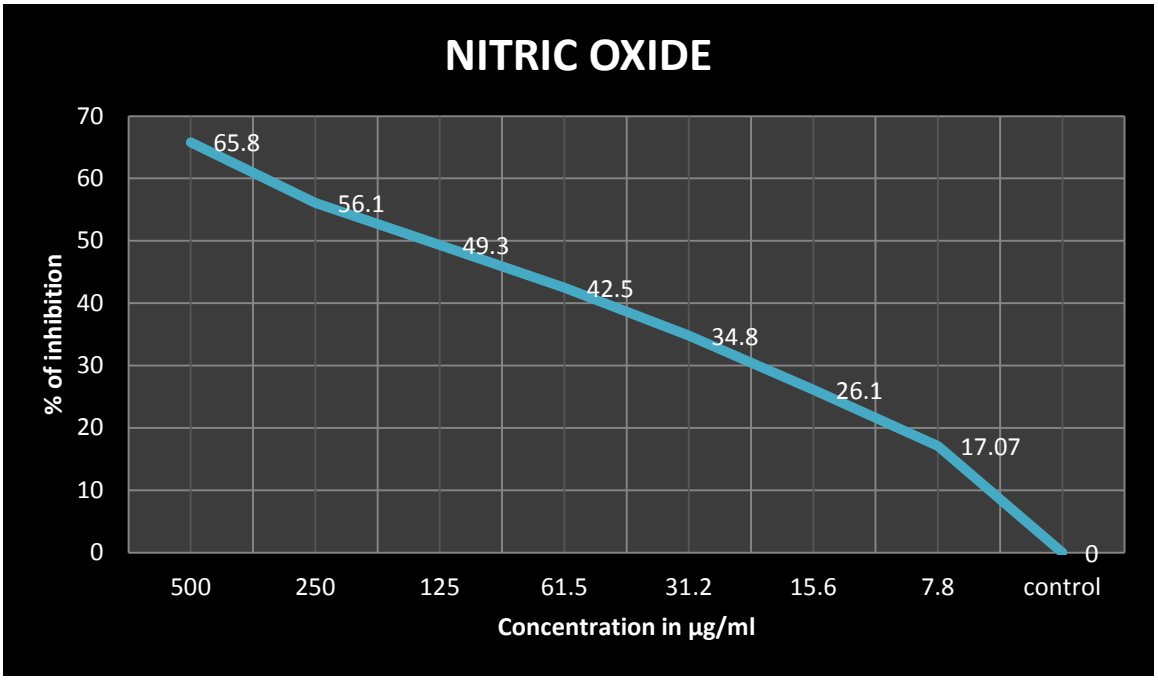


Fig no: 7 Nitric oxide radical scavenging assay IC50 values

iii) LIPID PEROXIDATION INHIBITION ASSAY:

The results of the ***in vitro*** antioxidant activity of Acetone fruit extract of vacciniumpallidum (F) Blue berry in lipid peroxide inhibition assay are given in (Table no : 3), The IC50 value of the standard (BHA) has been adopted from the previous studies (Huong et.al)

S.No	Concentration	% of inhibition	IC50 values µgm/ml
1	500	56.70±3.45 *	275±30 **
2	250	46.10±4.24	
3	125	29.80±6.8 **	
4	61.5	18.01±5.8	
5	31.2	12.08±4.81	
6	15.6	7.44±6.8 *	
7	7.8	1.0	
8	control	0.00	
9	Standard BHA	-	228

Table no 6: Lipid peroxidation assay of Aceton fruit extract of vacciniumpallidum (F) Blue berry

Values are the average of (n=3) and represented as mean ± standard deviation. IC50 values are not significant when compared with standard.

iv) Lipid peroxide inhibition assay:

The results of the *in vitro* antioxidant activity of Acetone fruit extract of vacciniumpallidum (F) Blue berry In lipid peroxide inhibition assay are given in (fig no : 6), The IC50 value of the standard (BHA) has been adopted from the previous studies (Huong et.al)

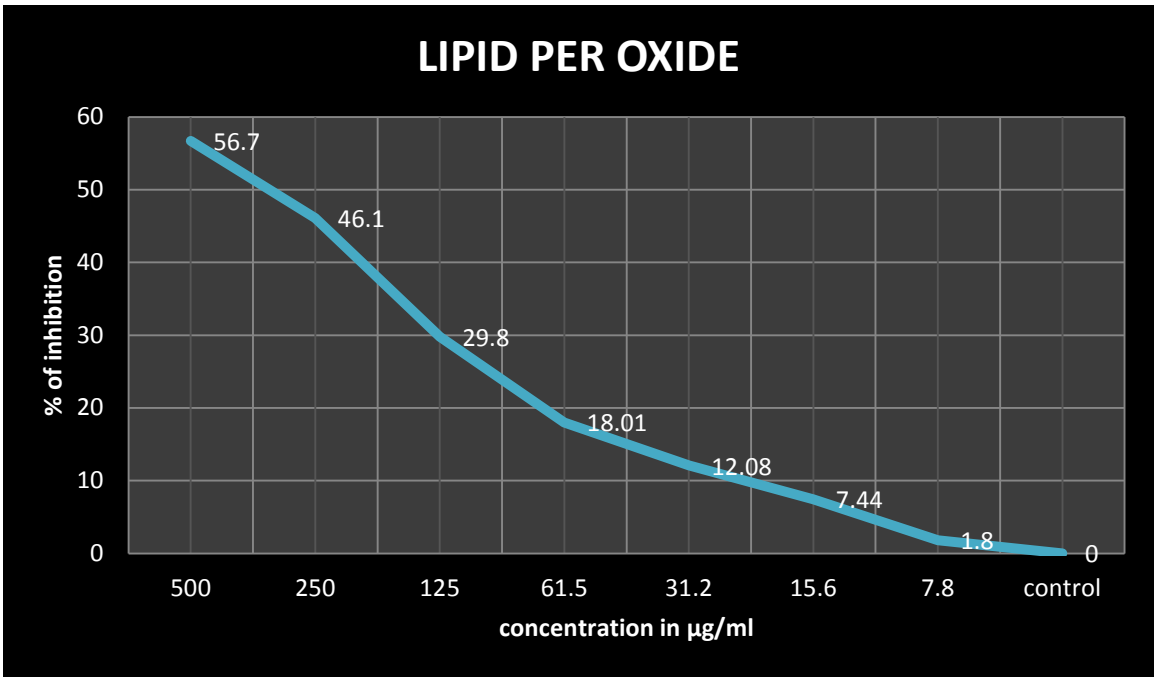


Fig no:8 Graphical representation of lipid peroxidation IC 50 values

v) Invitroanti cancer activity:

MTT Assay:

1. **Cytotoxic properties of test drug against HCT116 cell lines :**

The results ofIn vitro cytotoxic concentration of Aceton fruit extract of vacciniumpallidum (F) Blue berry.

MTT Assay method on HCT116cell lines .CTC 50 values are given in table no :4

S.No	Name of cell line	Test concentration	%of cytotoxicity	CTC 50 (µg/ml)
1		500	69.49±1.30	
2		250	52.21± 0.23 *	
3	HCT116	125	41.81 ±1.64	230±16.32*
4		62.5	31.91 ±14.73 *	
5		31.2	20.11± 4.21*	

Table no 11: CTC 50 value of Aceton fruit extract of vacciniumpallidum (F) Blue berry

Aceton fruit extract of Vacciniumpallidum (F) Blue berry:

Cytotoxic assay on HCT116 cell line:

The results of Invitrocytotoxic concentration of Acetone fruit extract of vacciniumpallidum (F) Blue berry in MTT Assay method on HCT 116 cell lines . CTC 50 values are given
infigno:29

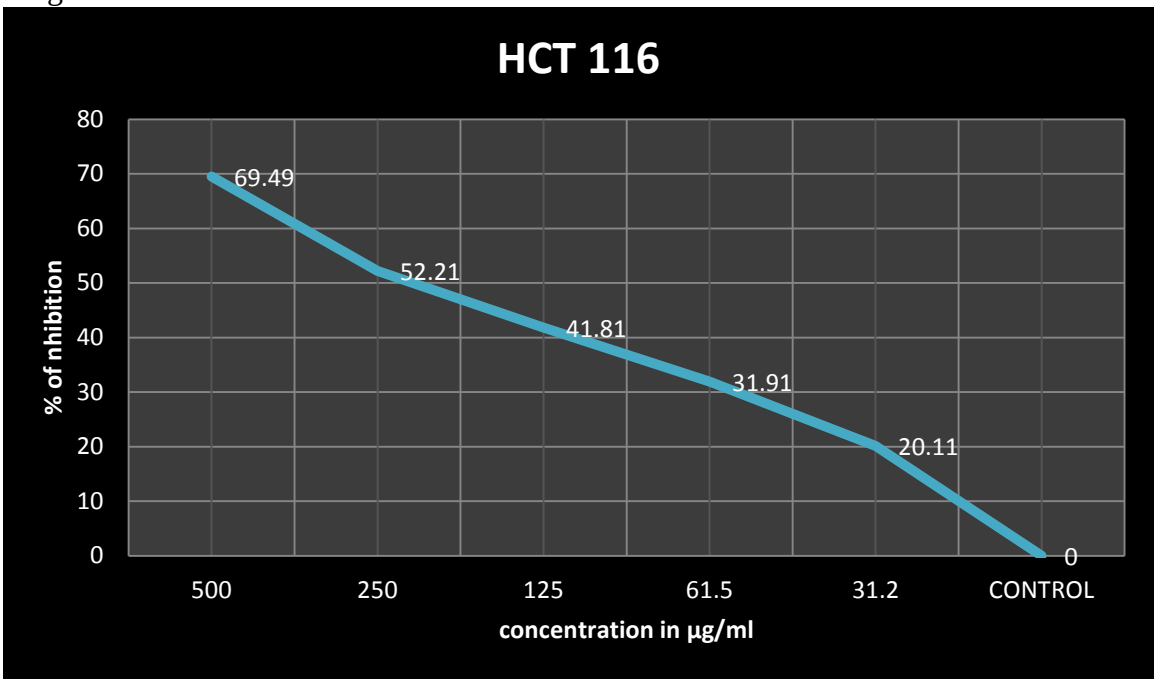


Fig no: 9 Graphical representation of cytotoxicity of Acetone fruit extract of vacciniumpallidum (F) Blue berry with CTC 50.

3. Cytotoxic properties of test drug against HT-29 cell lines :

The result of In vitro cytotoxic concentration of Acetone fruit extract of vacciniumpallidum (F) Blue berry in MTT Assay method on HT29 cell lines. CTC 50 values are given in table no: 5

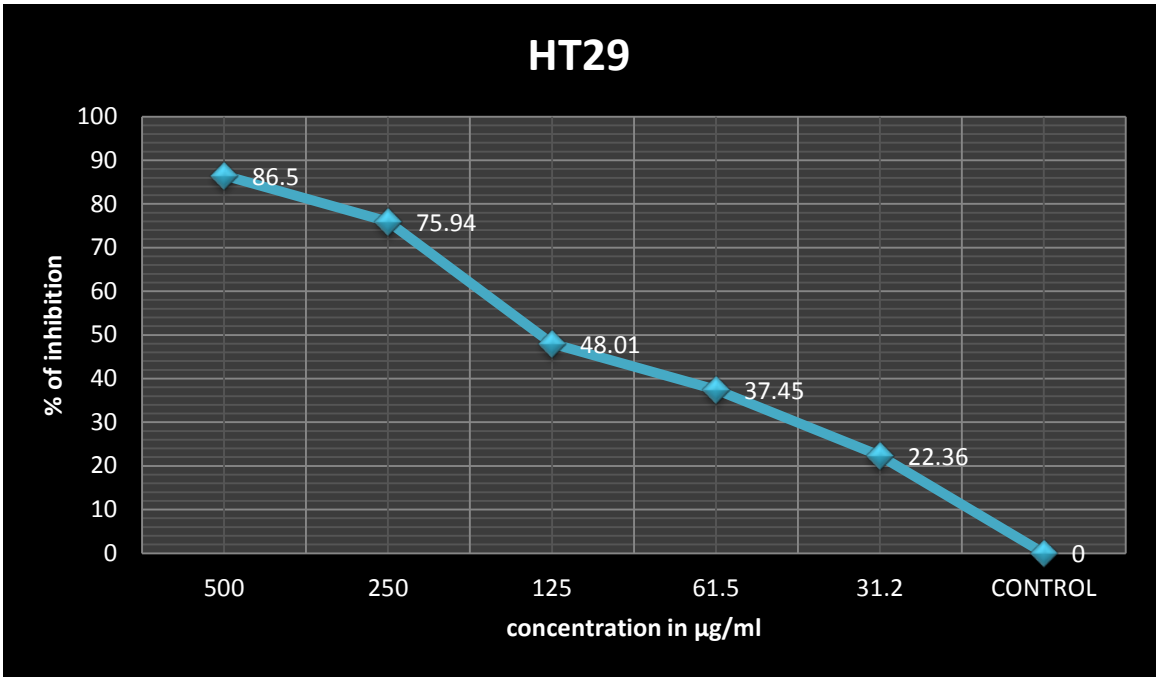
S.No	Name of cell line	Test concentration	%of cytotoxicity	CTC 50 (µg/ml)
1		500	86.50±0.86	
2		250	75.94±0.41*	
3	HT- 29	125	48.01±8.52	140.33±23.0**
4		62.5	37.45±9.87*	
5		31.2	22.36±4.54	

Table no 10 : CTC 50 value of Aceton fruit extract of vacciniumpallidum (F) Blue berry.

Acetone fruit extract of Vacciniumpallidum (F) Blue berry:

Cytotoxic assay on HT-29:

The result of In vitro cytotoxic concentration of ***Acetone fruit extract of vacciniumpallidum (F)*** *Blue berry* in MTT Assay method on HT29 cell lines. CTC 50



Values are given in figure no :31

Fig no: 31 Graphical representation of cytotoxicity of Aceton fruit extract of vacciniumpallidum (F) Blue berrywith CTC 50

Cytotoxic properties of test drug against HT115 cell lines :

The results of In vitro cytotoxic concentration of Acetone fruit extractof vacciniumpallidum (F) Blue berry inMTT Assay method on HT115 cell lines.

Sl.no	Name of cell line	Test concentration	% of cytotoxicity	CTC 50 (µg/ml)
1		**500**	**67.44 ±0.66**	
2		**250**	**33.32 ±2.83***	
3	**HT115**	**125**	**21.38 ±3.88**	**290 ±26***
4		**62.5**	**8.35 ±5.22***	
5		**31.2**	**6.11 ±7.07**	

Table no 6 : CTC 50 value of Acetone fruit extract of Vacciniumpallidum (F) Blue berry.

Acetone fruit extract of Vacciniumpallidum (F) Blue berry:

cytotoxic assay on HT115 Cell line:

The result of Invitrocytotoxic concentration of Acetone fruit extract of vacciniumpallidum (F) Blue berry in MTT Assay method on HT115 cell lines .

CTC 50 values are given in fig no :11

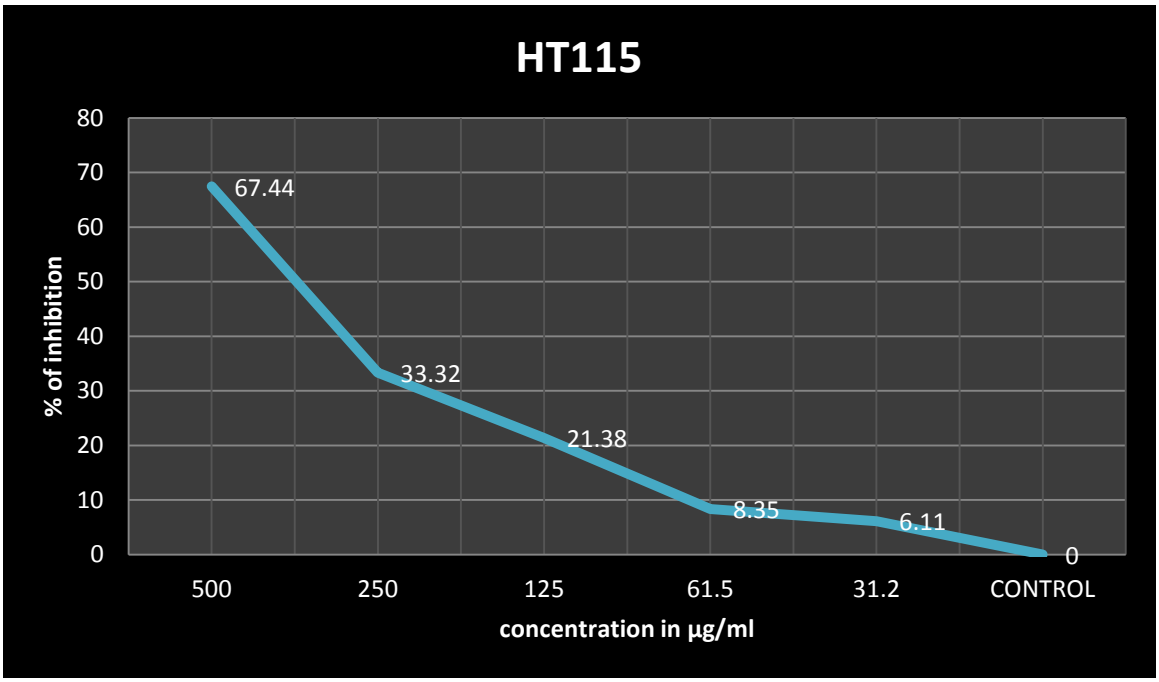


Fig no: 30 Graphical representation of cytotoxicity ofAceton fruit extract of vacciniumpallidum (F) Blue berry with CTC 50

VII. DISCUSSION

In this present study the phytochemical analysis of Acetone fruit extract of vacciniumpallidum (F) Blue berryshowed the presence of alkaloids, phenols, sterols, flavonoids, tannins, carbohydrates, Saponins, Triterpenoids which are well known bio active components. Flavonoids, Phenols are responsible for antioxidant activity, the amount of total flavonoids, phenols in the extract suggest that the extract possesses an antioxidant activity *Invitro.(*Zheng et.al*)* Flavonoids are group of poly phenolic compounds with unknown properties, which include free radical scavenging , inhibition of hydrolytic and oxidative enzymes. Phenolics are ubiquitous secondary metabolites in plants and possesses a wide range of therapeutic uses such as anti oxidant, anti mutagenic, anti carcinogenic, free radical scavenging activities. These also decrease cardiovascular complications. The scavenging ability of phenolics is mainly due to the presence of hydroxyl group.(Md. Ekramul Islam et.al)

The free radical scavenging activity of fruit extract of blue berry was evaluated based on the ability of quench the synthetic DPPH.Aceton fruit extract of vacciniumpallidum (F) Blue berry possessed significant DPPH radical scavenging activity (IC 50 Values 84±1.8) compared with standard rutin(IC 50 values 46.50±1.79).

Scavenging of nitric oxide compete with oxygen leading to the reduced production nitric oxide. Our finding suggests that the phenolic compounds present in the extract might responsible for nitric oxide scavenging effect. Hence the scavenging activity of vacciniumpallidum (F) Blue berry extract increases in dose dependent manner. The extract of Nitric oxide scavenging activity (IC 50 Values 66±1.6) compared with standard rutin (IC 50 Values 60±4.4)[P. Siva kumar et.al, Sujit etLipid peroxidation has been implicated in pathogenesis of number diseases including neurodegenerative diseases it is well established that bio enzymes are very much susceptible to LPO which is considered to be the starting point of many toxic as well as degenerative process. .Aceton fruit extract of vacciniumpallidum (F) Blue berry extracts inhibits the rate of lipid peroxidation by a reduction in red colour complex. It is generally assumed that ability of the plant phenolic compounds such as flavonoids to chelate ions in the LPO system is very important for their anti oxidant property.

Hence the extract possessed not significant lipid peroxidation inhibition activity (IC 50 Values 275±30) compared with standard BHA (IC 50 Values 228)

The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used. Cytotoxic concentration of .Aceton fruit extract of vacciniumpallidum (F) Blue berry different cell lines HCT116(CTC 50 Value 230±16.32), HT29(CTC 50 Value 140±23.0) HT-115(CTC 50 Value 220±26.0) was reported but extract was shown anti canceractivity..in colon cancer

All these observation s enabled us to confirm the anti oxidant, anti cancer activity of .Aceton fruit extract of vacciniumpallidum (F) Blue berry.

VIII.CONCLUSION

- The anti oxidant activity of Blue berry Fruit has been screened using DPPH assay, Nitric Oxide scavenging assay, Lipid Peroxidation assay, Total Antioxidant Capacity (TAC) and the values were found to be IC50: 41±1.8, 63±1.6,548±20,590.30µg/AE/gm of extract respectively.
- The anti cancer screening was performed using MTT assay using different cell lines(Vero, HCT116, HT115, HT29), CTC50 values were found to be >500, 230±16.32, 220±26,140±23.0.

To conclude, this study supports the contention that traditional medicines, dietary supplements and plant feed sources remain a valuable source in the potential discovery of natural product pharmaceuticals. The presented data .Acetone fruit extract of vacciniumpallidum (F) Blue berry having various activities such as anti oxidant, , and anti cancer activity support the folk information regarding of the plant. By using natural anti oxidants we can prevent, cancer, and and also we can prevent diabetic complications such as diabetic nephropathy, diabetic retinopathy, and diabetic cancer.

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